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Chlamydial and Rickettsial Infections

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I. INTRODUCTION

There is no more challenging dilemma than that presented to mammalian hosts by infection with chlamydiae and rickettsiae. These pathogens have adopted specialised mechanisms to assure satisfaction of

NATURAL IMMUNITY
ISBN 0 12 5145551

their metabolic deficiencies: bacterial replication, and therefore survival, can occur only within cells. Once inside the host, the intracellular infectious cycle is repeated as bacteria pass from infected cell to neighbouring cell, metabolically inert, and frequently disguised in host-derived material. Tissue destruction and pathology eventually follow, and are usually evident well before the host is immunologically aware of the invader. The dilemma for the host lies in the mechanisms at its disposal for elimination of sequestered intracellular parasites once awareness occurs. These mechanisms are few and, as we will discuss throughout the chapter, less than optimally effective.

The chlamydiae and rickettsiae are set apart from other prokaryotes by their exclusively intracellular habitat in infected experimental animals or incidental human hosts. The differences between these two taxonomic groups, however, are substantial, and necessitate a separate discussion of natural immunity based on the distinctive attributes of each.

II. CHLAMYDIAE

Chlamydia species are obligate intracellular prokaryotes that replicate exclusively within the confines of a membrane-bound cytoplasmic vesicle (inclusion) in susceptible eukaryotic host cells. The genus is comprised of two species, *C. psittaci* and *C. trachomatis*. Chlamydial cellular morphology and ultrastructure is identical for both species. The organisms possess an outer envelope similar to that observed for Gram-negative bacteria, and lipopolysaccharide (LPS) is a common genus antigen for the chlamydiae (Caldwell and Hitchcock, 1984; Nurminen *et al.*, 1983). Each species, and the strains or serovars within either species, can be distinguished by other outer envelope protein epitopes (Batteiger *et al.*, 1985).

The agent of psittacosis, *C. psittaci*, is widely distributed in nature, and causes acute disease and persistent infections in a variety of vertebrate and invertebrate hosts. Transmission of *C. psittaci* may occur via the respiratory route, by ingestion of infectious organisms, by direct contact with infectious organisms on mucosal surfaces, or transplacentally. *C. trachomatis* is host-restricted, in contrast, and humans are essentially the sole susceptible host species. Most *C. trachomatis* infections occur by direct contact of the organism with mucosal surfaces, either through sexual contact or by direct instillation into the conjunctival sac. Respiratory transmission has also been reported, but actually may involve nasopharyngeal colonisation followed by extension to the respiratory tree.

A. Intracellular Infection

Chlamydia psittaci infects a variety of host cell types, including mononuclear phagocytes and epithelial cells. This may contribute to the systemic spread of *C. psittaci* within the infected individual. *C. trachomatis*, in general, is much more host cell restricted; this organism causes productive infections only within mucosal epithelia. Thus, dissemination of *C. trachomatis* is more likely to occur by direct extension from an initial infectious focus. A notable exception is the lymphogranuloma venereum (LGV) biovar of *C. trachomatis*, which can cause productive infections within lymph-node macrophages. LGV infections also have the potential to cause systemic complications as a result of lymphatic dissemination.

1. Attachment of chlamydiae

The mechanism for attachment and uptake of chlamydiae into host cell cytoplasmic vesicles is similar for both chlamydial species. A specialised chlamydial developmental form (the elementary body, EB) mediates the initial interaction between chlamydiae and the host cell. Evidence for specific chlamydial ligands acting as adhesins for attachment to susceptible host cells has been reported (Byrne and Moulder, 1978), but a chlamydial adhesin molecule has not yet been identified (Hackstadt and Caldwell, 1985). The chlamydial adhesin binds to a host cell surface glycoprotein that has thus far been defined only on the basis of its sensitivity to protease (Byrne and Moulder, 1978) and neuraminidase treatment (Kuo *et al.*, 1973). This putative glycoprotein receptor may be localised to regions of the host cell membrane associated with coated pits (Hodinka and Wyrick, 1986), although this finding remains controversial (Ward and Murray, 1984). The attachment process requires no energy expenditure on the part of the chlamydiae. In fact, the EB is a metabolically inert particle. Host cell energy requirements also are not essential for attachment, since the process occurs both at lowered temperatures and in the presence of energy uncouplers (Moulder, 1985).

2. Entry of the chlamydiae

Entry of chlamydiae occurs rapidly after attachment, although the two steps can be temporally separated (Byrne, 1978). The process resembles phagocytosis, and requires energy expenditure by the host cell. Chlamydiae do not participate metabolically, yet they do direct the process; the putative chlamydial adhesin must initiate the circumscription of the tightly opposed host cell membrane about the chlamydial EB.

3. Intracellular localisation of the chlamydiae

Once the entry process has been effected, the chlamydiae remain within the endocytic vesicle and undergo differentiation from the sturdy, metabolically inert infectious EB to a larger, osmotically sensitive reticulate body, or RB (Hatch *et al.*, 1984; Newhall and Jones, 1983). Intracellular replication does not begin until differentiation of the EB to the RB is complete. In addition, chlamydiae inhibit the fusion of lysosomes with chlamydiae-containing endocytic vesicles, and are thereby protected from this potent host cell defence mechanism (Eissenberg and Wyrick, 1981; Friis, 1972).

RB growth and division proceeds by binary fission within the endocytic vesicle. Vesicle membrane is added as the replicating microcolony increases in size, but the mechanism for inclusion growth has not been well characterised. Chlamydial growth proceeds for a length of time that is dependent upon both the chlamydial strain and the host cell type. Eventually, nearly the entire host cell cytoplasm is filled by the growing inclusion body. At this point a second round of differentiation ensues, and RBs differentiate to infectious EBs. The signal that triggers this event is not known, but may depend upon either depletion of some essential nutrient or accumulation of toxic metabolic products. Soon after RB to EB differentiation, the inclusion membrane disrupts. This is followed by plasma membrane lysis: a new population of EBs are released, each one capable of initiating an infectious cycle. Under ideal conditions in vitro, an increase of about [illegible] of chlamydiae occurs over the input multiplicity, and the process takes from two to three days to complete.

4. Progressive infection

The chlamydial developmental cycle has been well characterised in cell-culture systems, and is believed to proceed in a similar manner during actual chlamydial infections. Infections of mucosal surfaces (oculogenital infections) tend to remain localised at the initial infectious focus, but may spread by direct extension. For example, infections of the endocervix may directly ascend the genital tract resulting in uterine (endometritis) and fallopian tube (salpingitis) infections. It is not clear if mononuclear phagocytes can serve as reservoirs for chlamydial persistance, but overt intracellular development of *C. trachomatis* has not been characterised in these cells.

B. Natural Immunity

Chlamydial growth and development, as detailed above, must proceed according to six well-defined steps: (a) attachment; (b) entry; (c) organisation of infectious to replicative forms; (d) multiplication; (e)

reorganisation of replicative forms to infectious forms; and (f) release of infectious forms from the host cell. In addition, the chlamydiae, like all transmissible pathogens, must be effectively passed from one host to another. Interruption of any one of these essential functions by a host not previously exposed to chlamydiae would be considered natural immunity, a response that does not rely on acquired immune reactions such as development of chlamydia-specific antibodies. Natural immunity will be discussed under the broad headings of barrier mechanisms, acute-phase reactions (such as inflammation and complement activation), and non-specific cytokine-mediated defence mechanisms. Although the contribution of both non-specific and acquired immunity in the control of diseases caused by the chlamydiae has been demonstrated, it is important to recognise that immunity does not necessarily lead to eradication of the organisms. All chlamydial infections tend to persist if left untreated. The mechanisms involved are by no means clear, but certain changes that accompany resolution of acute chlamydial disease may contribute to persistent or chronic infections. Indeed, immune responses, both natural and acquired, may in large part be responsible for the pathology of acute disease, as well as progression to chronicity.

1. Barrier mechanisms

Most successful pathogens have evolved survival strategies to either penetrate through or avoid undamaged skin, a formidable natural protective barrier. Certain pathogens, like the staphylococci and other opportunists, gain entry into the host via breaks or lesions in the skin. The rickettsiae and many parasitic protozoans and nematodes are injected directly by the bite of an insect. Other potential pathogens are ingested, and either cause gastrointestinal disease or disseminate from an initial infectious focus in the gut. Chlamydiae, on the other hand, most frequently become associated with hosts at mucosal surfaces, and are often transmitted by the intimate, frequently prolonged contact between mucosae associated with sexual activity. Transmission of chlamydiae can also occur by direct instillation of organisms in the conjunctival sac or by droplet inhalation into the respiratory tree. In each instance, however, barrier mechanisms at the mucosal surface represent the initial obstacle that must be overcome for chlamydiae to survive and replicate. The ciliary flow of mucus is a powerful barrier to the establishment of an infectious focus. Specific attachment of chlamydiae to respiratory, conjunctival, or genital epithelia, and subsequently parasite-specified entry into susceptible host cells may overcome the natural barriers at mucosal surfaces. Nonetheless, transmission from infected individual to cohort is less than fully efficient, and these barrier mechanisms may play a role in natural resistance to chlamydiae by limiting successful transmission.

2. Acute-phase reactions

Several *C. trachomatis* strains activate complement by the alternate pathway (Megran *et al.*, 1985). High levels of C5a are found in normal plasma following exposure to chlamydial EBs, and chlamydiae-mediated activation of the complement cascade results in the stimulation of polymorphonuclear leukocyte (PMN) chemotaxis. The presence of PMNs is characteristic of the initial inflammatory response to virtually all chlamydial infections, and PMNs can ingest and kill chlamydial EBs in vitro by mechanisms apparently unrelated to the production of toxic oxygen metabolites (Yong *et al.*, 1982). It is not clear how effective the PMN and chlamydiae interaction is in vivo, however, since infectious foci are maintained even in the presence of a profound PMN-predominant inflammatory response. As infections proceed from acute to chronic stages, the cellular infiltrate matures from predominantly PMN to predominantly mononuclear in character. In this progression, chlamydiae may (1) be protected from immune reactive cells by virtue of their intracellular habitat within mucosal epithelia, (2) be taken up and either persist or be killed by PMNs or mononuclear phagocytes, or (3) be inhibited from replicating or killed by host cells altered directly by soluble mediators (cytokines) released from lymphocytes or other cells attracted to the initial focus of infection. It is in these host–chlamydia interactions that natural immunity can contribute to the resolution of acute chlamydial disease. The net result of these early events associated with chlamydial infections could be either eradication of the pathogens or development of persistent infections. Each of these outcomes may occur under different circumstances; unfortunately, the circumstances that govern eradication or persistence are not well understood at the present time.

3. Cytokine-mediated defence mechanisms

Chlamydiae are nonviral microbial inducers of interferon, and like many of the other intracellular pathogens, their growth is inhibited in cells treated with interferon (IFN), especially gamma interferon (IFN-γ). Spleen cells from experimental animals infected with chlamydiae secrete much higher levels of IFN when stimulated in vitro with either specific antigen or mitogens than do cells from control animals (Byrne and Faubion, 1982). The influence of interferons on intracellular chlamydial growth has been well studied in cell-culture systems, but it is not clear yet how interferons actually influence the course of naturally occurring chlamydial infections. The effects of cytokines on chlamydiae appear to be restricted to events that occur after entry into host cells: cytokines do

not influence either the attachment or phagocytosis of chlamydia, but do interrupt chlamydial growth and development within treated cells (Byrne and Faubion, 1982). All classes of interferons inhibit the intracellular development of *C. trachomatis*, but the degree of inhibition is at least partially dependent on the interferon class, the particular chlamydial strain, and the host cell type (Byrne, 1986; Byrne and Rothermel, 1983; de la Maza *et al.*, 1985). The mechanism of interferon-mediated inhibition of growth is not known, but has been reported for IFN-treated monocytes, macrophages, fibroblasts and other somatic cell types (Byrne and Krueger, 1983: Shemer and Sarov, 1985). No evidence for oxygen-dependent killing has been found, and some reports suggest that oxygen-independent mechanisms result in curtailment of replication that promotes chlamydial persistence (Byrne and Faubion, 1983; Byrne *et al.*, 1986; Murray *et al.*, 1983). Although the precise mechanism for IFN-mediated inhibition of *C. trachomatis* replication is not well understood (de la Maza *et al.*, 1985), inhibition of *C. psittaci* replication in cells treated with IFN may be related to the induction of indoleamine-2, 3-dioxygenase, and the resultant depletion of tryptophan, an essential amino acid (Byrne *et al.*, 1986). This mechanism has been reported for IFN-mediated inhibition of intracellular *Toxoplasma gondii* replication in fibroblasts (Pfefferkorn, 1984): *T. gondii* is an obligate intracellular protozoan that also lives within membrane-bound cytoplasmic vesicles that do not fuse with lysosomes.

Thus, the cytokine system, an important natural immune defence mechanism against many intracellular pathogens, may serve to restrict intracellular chlamydial replication within a variety of host cells without actually eradicating the organisms. IFN-mediated cytostasis may, in fact, contribute to immune-mediated chlamydial persistence rather than non-specific protection.

The essential stages of chlamydial development and possible points at which various parameters associated with natural immunity could act to interrupt the infectious process are outlined in Table 19.1.

C. In vivo Infections

1. Experimental animal models

A variety of experimental animal models have been developed to study natural and acquired immunity to *C. psittaci*, but mice and guinea pigs have been widely used for analysis of both systemic and local mucosal infections (Howard *et al.*, 1976; Lammert and Wyrick, 1982; Meyer and Eddie, 1962; Rank *et al.*, 1985a). The route of *C. psittaci* inoculation into

Table 19.1: Essential stages of chlamydial growth and points where parameters of natural or acquired immunity may interrupt the infectious cycle

Growth stage	Natural immunity parameter	Acquired immunity parameter
Attachment of EB[a] to susceptible host cell	Barrier mechanisms, especially mucociliary flow	Neutralising or opsonic antibody
Endocytosis of EB	Complement, chemotactic induction of PMNs and other inflammatory cells resulting in ingestion by non-permissive host cell	Opsonisation
Differentiation of EB to RB[b]	?	?
Multiplication of RB	Cytokine system (IFN etc.); inhibition of RB growth in activated host cell	Cytotoxic cells if novel antigens expressed on infected cell surface; augmented cytokine system
Differentiation of RB to EB	?	?
Lysis of infected host cell and re-infection	?	Neutralising or opsonic antibody
Transmission to new host	Barrier mechanisms	Neutralising secretory antibody

[a] EB = elementary body, the infectious form of the organism.

[b] RB = reticulate body, the intracellular replicative form of the organism.

mice profoundly influences the course of subsequent disease: systemic introduction of a large inoculum of viable chlamydiae results in a rapidly lethal infection that is attributed to a putative chlamydial toxin. No toxic factor other than LPS has ever been associated with chlamydiae, however. Mouse toxicity is inhibited by immunisation either with killed organisms or with live organisms introduced subcutaneously in low numbers (Bell *et al.*, 1959). Since LPS is a genus-specific antigen, and prevention of toxicity is strain-specific, it is doubtful that LPS alone mediates this toxic response. Immediate toxicity may be the result of chlamydia-induced chemotaxis and degranulation of PMNs, with resulting vascular shock (Moulder *et al.*, 1976). Chlamydial inocula introduced locally, either at mucosal surfaces (conjunctiva, genital tract) or subcutaneously, can result in the induction of immunity. This acquired immunity may confer protection against subsequent infections, although in most experimental systems this protection is quite relative and short-lived.

Chlamydia trachomatis is much more difficult to study than *C. psittaci* in experimental animal model systems. The organism is primarily a human pathogen: with the exception of some primate systems (Harrison *et al.*, 1979), useful animal models that reproduce human disease have been a research area that has remained undeveloped. Recently, both systemic (Brunham *et al.*, 1985) and mucosal (Tuffrey *et al.*, 1982) murine models have been reported. Although disease spontaneously resolves in these infected mice, inapparent infection persists; infected animals treated with immunosuppressive drugs exhibited recrudescence of disease after apparent resolution of the primary infection (Stevens *et al.*, 1982). Since infections can be established with human chlamydial strains in both primates and mice, appropriate host cell receptors must be present on mucosal epithelia of these species. A murine chlamydial strain that shares DNA homology and species-specific antigens with *C. trachomatis* has also been used extensively (Barron *et al.*, 1984, 1981; Rank *et al.*, 1985b; Swenson *et al.*, 1983; Williams *et al.*, 1981, 1984a, 1984b).

2. Human infections

Chlamydia trachomatis is the most common sexually transmitted pathogen in both Great Britain and the United States. It is responsible for a significant portion of uncomplicated urethritis cases in males, and also has been associated with complications such as epididymitis and proctitis. In females, *C. trachomatis* causes cervicitis, but the major health risk associated with this organism is a result of ascending complications of cervical infections. These include endometritis and salpingitis (pelvic inflammatory disease) with the attendant risk of infertility. *C. trachomatis* is also transmissible to the neonate during passage through an infected

cervix; inclusion conjunctivitis and a distinctive pneumonia are the major chlamydial diseases of the newborn. Trachoma, a result of repeated chlamydial ocular infections over a period of years, remains the leading cause of preventable blindness in the world today (Oriel and Ridgway, 1984; Schachter and Dawson, 1978). Undefined species-specific innate factors play a role in natural immunity to *C. trachomatis*, since humans are uniquely susceptible to infection by this organism. The nature of these factors, however, remains a mystery. *C. psittaci*, in contrast, is not host range restricted, although subspecies host range specificity cannot be excluded.

III. RICKETTSIAE

The individual rickettsial species within the order Rickettsiales are genetically (Schramek, 1972; Tyeryar *et al.*, 1973; Myers and Wisseman, 1980), morphologically (Plotz *et al.*, 1943; Silverman and Wisseman, 1978) and physiologically (Weiss, 1973) distinct. Nonetheless, as a group they share two major characteristics: (a) they are all symbionts or parasites of arthropods, which serve as vectors for transmission; and (b) they are all obligate intracellular parasites of eukaryotic cells of their incidental mammalian hosts. These characteristics provide the rationale for the presently accepted rickettsial taxonomy (Table 19.2 and

Table 19.2: Classification of the rickettsiae[a]

Organism	Vector	Natural host	Clinical syndrome
Tick-borne rickettsiae:			
R. rickettsii	tick	rodents/vertebrates	Rocky Mountain spotted fever
R. conorii	tick	rodents/vertebrates	Boutonneuse fever
R. sibirica	tick	rodents/vertebrates	North Asia tick typhus
R. australis	tick	rodents/vertebrates	Queensland tick typhus
R. akari	tick	rodents	Rickettsialpox
Insect-borne rickettsiae:			
R. typhi	flea	rats/mice	Endemic (murine) typhus
R. prowazekii	louse	human	Epidemic typhus
Trombiculid-borne rickettsia:			
R. tsutsugamushi	chigger (mite larva)	mammals	Scrub typhus

[a] Classification according to vector (as in this table) is supported by both immunochemical and genetic relationships between individual species of rickettsiae pathogenic for humans within the vector groups (Marchette, 1982).

Marchette, 1982). The recent Class III biological safety classification of all the rickettsiae has restricted experimental analysis of these micro-organisms to a few research centres; each centre concentrates on one or a very few species. Although some trends have emerged, the more frequent striking differences between species caution against broad generalisation across the taxonomic group.

Transmission of the rickettsiae in nature is exclusively through bites of arthropod vectors, and epidemiology of the rickettsioses suggests that host range is determined by vector habitat. For example, *R. prowazekii*, aetiologic agent of epidemic typhus, is transmitted from person to person by the human body louse. To date, few non-human mammalian reservoirs for this particular strain of rickettsiae are reported (Bozeman *et al.*, 1975; Marchette, 1982). In contrast, *R. tsutsugamushi*, aetiologic agent of scrub typhus, is transmitted by the bite of the larvae of trombiculid mites. *R. tsutsugamushi* can be isolated from many different mammalian species in the natural environment of the mite, as well as from incidental intruders into the habitat (Marchette, 1982).

The probable target cell in vivo for all the rickettsiae is the endothelial cell: rickettsial invasion of endothelium appears to cause the initial pathology and symptoms of infection. Virtually every cultured cell type, however, will support replication of rickettsiae in vitro, including several reptile, insect, and avian cell lines. Experiments with infected animals suggest that inflammatory cells and cells of the immune system also become infected, and may provide the means for passage of rickettsiae to their vectors in the natural life cycle of these arthropods (Boese, 1972).

A. Intracellular Infection

The characteristic feature of rickettsiae that sets them apart from other free-living micro-organisms is the absolute requirement of these parasitic bacteria for an intracellular site of replication. Clearly, the satisfaction of such an important function, survival of the species, is an evolutionary imperative. Mammalian hosts have undergone a similar and parallel evolutionary pressure to assure homeostatic balance in their complex tissues that sustain life. The creativeness of the rickettsiae in their adaptation to the perils of intracellular existence is remarkable. The association of rickettsiae and humans is relatively young on an evolutionary scale, and only time will tell whether this association will stabilise into a symbiotic relationship that benefits survival of both populations. The measure of the instability of this relationship at present is the degree of pathology generated in the battle between host and parasite: in most instances, the rickettsiae appear to have the upper hand.

1. Attachment of rickettsiae

The ability of rickettsiae to infect a wide variety of cells, from the highly phagocytic neutrophil and macrophage to the barely phagocytic fibroblast and lymphocyte, argues against a specific receptor on cells for rickettsial attachment. In fact, studies with *R. prowazekii* suggest that the primary molecule to which rickettsiae adhere on red blood cells (RBCs) is cholesterol, or a complex of cholesterol and another unidentified molecule (Ramm and Winkler, 1976). Use of abundant membrane constituents for attachment would certainly explain the wide range of cells entered by rickettsiae. Despite the logic of such a ubiquitous attachment site, however, the idea that cholesterol is the receptor on cells other than RBCs has eluded confirmation (Winkler, 1986).

2. Penetration of rickettsiae

Entry of rickettsiae into cells is by 'induced phagocytosis', a process observed for many, if not all, obligately intracellular micro-organisms (see also Section II.A). Unlike that of chlamydiae, however, entry of rickettsiae requires the expenditure of energy by both the bacterium and the host cell (Cohn *et al.*, 1959; Walker and Winkler, 1978): for productive infection to occur, both the rickettsiae and the host cell must be metabolically active. Further, the host cell must be capable of phagocytosis: cells treated with cytocholasin B do not ingest attached rickettsiae (Ramm and Winkler, 1976; Winkler and Miller, 1982), nor do rickettsiae enter cells (RBCs) that are not phagocytic (Ramm and Winkler, 1976). Although the mystery of how rickettsiae facilitate their own uptake into cells remains unsolved, one interesting theory of the induction of phagocytosis is based on a phospholipase A_2 activity observed in the lysis of RBCs. Winkler (1986) has proposed a concerted process of induced phagocytosis in which attachment of rickettsiae to cells activates a phospholipase A activity (presumed to be on the rickettsiae) that releases fatty acids from the cell membrane, and signals the cell to internalise the damaged membrane for repair. The rickettsiae attached to this portion of the damaged cell membrane is phagocytosed as a bystander of the repair process, and continues the disruption of the membrane as it is internalised. As the process of phagocytosis is completed, so is membrane lysis at the distal end of the phagosome. Thus, theoretically at least, as the rickettsiae are being phagocytosed, they are simultaneously disrupting the phagocytic vacuole and releasing themselves into the cytosol of the cell. The host cell membrane retains its integrity since the phagocytic event has isolated the damaged portion of the membrane. This theory ties together several different observations,

and takes into account the information that certain of the rickettsiae have never been observed in a phagocytic vacuole, even within minutes of attachment and entry. However, the majority of data that contribute to this theory are from studies with *R. prowazekii*. Observations with the phospholipase A activity of *R. rickettsii* have been less consistent (Walker, 1984; Silverman, 1986). Moreover, *R. tsutsugamushi* has a complex, but markedly different scenario of entry into cells (discussed in Section III. A.4).

3. Intracellular localisation of the rickettsiae

The rickettsiae are energy parasites that can satisfy their metabolic needs only by localising in intracellular compartments rich in nutrients of the energy cycles. The major metabolic deficiency of typhus and spotted fever rickettsiae may be the generation of sufficient quantities of adenosine triphosphate (ATP) to fulfil all of their energy-requiring functions (Bovarnick, 1956). Given an exogenous source of glutamate, the rickettsiae can synthesise ATP *de novo*, albeit relatively poorly compared with other free-living bacteria (Bovarnick and Allen, 1957). The rickettsiae can, however, transport preformed ATP across their cell membrane, a process unusual among the prokaryotes (Winkler, 1976). The requirement for preformed ATP may explain the localisation of certain rickettsial strains, notably *R. tsutsugamushi*, in the glycogen- and mitochondrion-rich region of cells (Rikihisa and Ito, 1980). Although synthesis of ATP is minimal in *R. prowazekii*, pathways for liberation of energy by conversion of ATP to ADP are intact. Rickettsia-generated ATP is undoubtedly used to maintain baseline metabolic levels during transport from cell to cell, and for the energy required to induce phagocytosis. Thus, the rickettsiae can survive for short periods of time in an extracellular environment. They must, however, gain access to the intracellular environment of a cell for the preformed ATP to fuel such energy-requiring events as replication.

The intracellular location of the rickettsiae is characteristic for each species: *R. tsutsugamushi* is found only within the cytoplasm of the cell, generally in the perinuclear region; *R. typhi* and *R. prowazekii* are found dispersed throughout the cytoplasm; and the spotted fever group rickettsiae are found in both the cytoplasm and the nucleus of infected cells (Burgdorfer *et al.*, 1968) (Table 19.2). All rickettsiae multiply freely in the cytosol of the cell, and, in contrast to the chlamydiae, are not bound by any host-cell-derived membranes. The replication of rickettsiae is that charcteristic of all bacteria: division by binary fission. The generation time for each of the rickettsiae is quite slow, 8–20 hours, and

is influenced primarily by the nature of the host cell. Thus, a single rickettsial species will exhibit varying generation times in different cell lines (Wisseman *et al.*, 1976; Silverman and Bond, 1984).

4. Progressive infection

It is clear from studies on plaque formation by the different rickettsiae in tissue culture that all the rickettsiae spread from an initial infectious focus to adjacent cells. For certain of the rickettsiae, such as *R. prowazekii*, it is presumed that lysis of the originally infected cell precedes infection of neighbouring cells. The temporal residence of other rickettsiae within cells appears to be governed by internal signals within the micro-organism: shortly after the onset of intracellular replication (10–15 hours for *R. rickettsii*; 18–24 h for *R. tsutsugamushi*) one or more rickettsiae begin migrating from the original infected cell to establish infection in adjacent cells. The percentage of infected cells increases linearly with time in culture in the absence of any detectable cell loss (Wisseman *et al.*, 1976; Nacy and Meltzer, 1979; Silverman and Bond, 1984). The signals that regulate this early rickettsial migration are not known, but may confer an evolutionary advantage on the organism able to establish widespread infection without massive tissue destruction. How is migration of rickettsiae prior to cell lysis accomplished? For *R. rickettsii*, whose intracellular residence includes both the cytoplasm and the nucleus, there is little information. *R. tsutsugamushi* infection of mouse mesothelium in vivo, however, demonstrated an interesting, and possibly unique, mechanism by which this rickettsia passes from cell to cell (Ewing *et al.*, 1978). Egress from infected cells occurs by a budding process analogous to that of enveloped viruses, and the rickettsiae leave the cell surrounded by a host-derived plasma membrane. Ingestion by adjacent cells occurs by phagocytosis of this membrane-bound micro-organism, and rickettsiae are then found within the newly infected cell with two layers of host material. Within minutes of internalisation, the rickettsiae dissolve the two host membrane layers and are free in the cytoplasm to begin replication. Lysosomal fusion does not occur during this brief time. Thus, *R. tsutsugamushi* is not only protected from the potentially hostile extracellular environment by a coat of host cell membrane, but it is also protected from the major intracellular source of antimicrobial activity, the phagolysosome, by its rapid escape into the cytoplasm.

At some point late in the replication cycle of all rickettsiae, infected cells become so filled with bacteria that rupture occurs, releasing massive numbers of these micro-organisms, each one capable of initiating a new infectious cycle.

B. Natural Immunity

As with chlamydiae (see Section II.B), the interaction of rickettsiae and host cell proceeds in a well-defined, and logical sequence: (a) attachment; (b) entry; (c) replication; and (d) release from the cell. Unlike that of the chlamydiae, however, transmission of rickettsiae from the infected individual is *not* under the control of the host, but is accomplished almost exclusively through the natural life cycle of the various arthropods that serve as reservoirs and vectors. Natural immune parameters are most effective, therefore, during transmission of the rickettsiae from cell to cell, rather than from host to host. Despite powerful evidence in experimental animals that non-specific immunity plays a role in both the initiation of disease and the resolution of established infections, this area of host defence is relatively unexplored in humans. With the exception of rickettsialpox (aetiologic agent, *R. akari*), intervention by drugs such as tetracycline and chloramphenicol is recommended to reduce the mortality (20 to 60 per cent) of untreated rickettsial diseases. Even with drug treatment, there is ample evidence to suggest that rickettsial organisms persist in tissues for years in the absence of any clinical symptoms (Fox, 1948; Murray *et al.*, 1951). In these infected individuals a balance is achieved between factors that protect the host from overt disease and factors that favour survival of the rickettsiae: the regulation of this new homeostatic balance is as yet unclear. The concept of non-sterile immunity in rickettsioses, however, will be a fascinating intellectual challenge to unravel.

1. Barrier mechanisms

The integrity of skin is a major barrier used as natural protection against infection. Each of the rickettsiae successfully breaches this barrier by a vector mode of transmission, although introduction of the organism differs for the different rickettsial species. Scrub typhus and spotted fever rickettsiae are inoculated directly into extravascular spaces during the feeding cycle of the chigger and the tick, respectively. In contrast, participation of the host is required for infection by typhus rickettsiae: inoculation occurs by introduction of infected louse faeces when the host scratches the insect bite (Blanc and Baltazard, 1938). Hypersensitivity to the bite of arthropod vectors occurs with repeated exposure, and inflammatory cells which accompany hypersensitivity responses, rather than providing a second line of defence, appear to support survival and replication of the rickettsiae (Wisseman and Tabor, 1964; Gambrill and Wisseman, 1973a, b; Rikihisa and Ito, 1980; Nacy and Osterman, 1979; Turco and Winkler, 1984; Nacy and Meltzer, 1984).

2. Acute-phase reactions

The efficiency of rickettsiae for establishment of infections in vivo is astonishing. In experimental animal models, one lethal dose equals one infectious dose: that is, a single viable rickettsia is sufficient to establish a lethal infection in a susceptible host (Groves and Osterman, 1978). This incredible efficiency suggests that most of the interstitial barrier mechanisms available to the host (acute-phase proteins, alternate pathway complement activation, inflammatory cell infiltrates) will be less than effective in preventing establishment of an infectious focus. Once the rickettsiae are intracellular, the host is faced with a whole new set of problems. How then are rickettsial infections controlled? Mortality rates (considerably less than 100 per cent for each of the human rickettsial diseases), persistence of rickettsiae in tissues after resolution of primary infection, and experience with a number of experimental animal models, all suggest that unidentified innate host factors do contribute to the containment of rickettsial infections.

3. Cytokine-mediated defence mechanisms

Experimental evidence suggests that the resolution of primary disease is through the cell-mediated arm of the immune system: antigen-reactive lymphocytes, their soluble products (lymphokines), regulatory cytokines from non-immune cells, and non-specific effector cells such as activated macrophages and NK cells. The combination of cells and cytokines required to protect animals from each of the rickettsial species is likely to be different, but in each case, events that lead to eradication of rickettsiae or suppression of rickettsial growth can be divided into events that occur outside of cells, and events that occur inside.

a. Factors that affect attachment or entry of rickettsiae

Under certain well-defined experimental conditions, the interaction between rickettsiae and macrophages during evolution of an immune response regulates susceptibility to disease (Nacy and Groves, 1981; Nacy and Meltzer, 1984; Jerrells and Osterman, 1981, 1982a). An analysis in vitro of the factors that play a role in this non-specific macrophage-mediated resistance demonstrates that the events for acquisition of antirickettsial activity by macrophages can be temporally separated into two discrete phases: (1) macrophages exposed to lymphokines (the soluble products of antigen-stimulated lymphocytes) before interaction with rickettsiae develop the capacity to resist infection with the bacterium; and (2) macrophages exposed to lymphokines after rickettsiae establish an intracellular infection acquire the capacity to kill

the intracellular parasite (Nacy and Meltzer, 1979). The lymphokine-induced decrease in the ingestion of rickettsiae is fascinating, and has now been documented with several different rickettsiae (Nacy and Meltzer, 1979; Nacy and Meltzer, 1982; Turco and Winkler, 1984), and a variety of other obligate and facultative intracellular parasites (Miller and Twohy, 1969; Salvin and Chang, 1971; Hoff, 1975; Nacy *et al.*, 1981; Horwitz and Silverstein, 1981; Pappas and Nacy, 1983). This antimicrobial activity is not simply an alteration in phagocytic capacity of macrophages, nor is it a reflection of toxicity of lymphokines for these parasites (Nacy *et al.*, 1981; Oster and Nacy, 1984). The actual mechanism behind this reduction in the number of infected macrophages is not known but, since it is not parasite-specific, it is likely to be associated with an extracellular killing mechanisms at the site of parasite attachment.

The regulation of macrophage resistance to infection is an example of an effector reaction that is induced by the co-operation of several distinct lymphokines: both IFN-γ and another non-IFN macrophage-activating lymphokine are required for the reaction to proceed; neither activating factor alone is effective (Meltzer *et al.*, 1986). That this effector reaction develops during in vivo infections has been documented with both *R. tsutsugamushi* and *R. akari* (Nacy and Meltzer, 1982, Nacy *et al.*, 1983; Nacy and Meltzer, 1984).

b. Factors that affect intracellular survival of rickettsiae

Macrophages treated with lymphokines after infection with rickettsiae develop the capacity to kill the intracellular bacterium (Nacy and Meltzer, 1979; Wisseman and Waddell, 1983; Turco and Winkler, 1984; Jerrells *et al.*, 1986). The lymphokines that induce this effector reaction are several, and may differ for the different rickettsial species (Nacy *et al.*, 1981): without question, IFN-γ is the major, and in some case the only, macrophage-activating agent. For *R. tsutsugamushi*, both IFN and non-IFN-γ activating factors are effective in induction of intracellular killing by macrophages (Nacy *et al.*, 1981): these lymphokines act independently, but may co-operate by affecting different macrophage subpopulations for maximal intracellular killing in vivo (Nacy *et al.*, 1985). For intracellular destruction of *R. conorii*, however, IFN-γ may be the only lymphokine that regulates this macrophage-mediated effector activity: cloned murine IFN-γ reproduced the activity of lymphokines for induction of rickettsiacidal activities against *R. conorii* in macrophage monolayers, and monoclonal antibodies prepared against IFN-γ abrogated lymphokine-induced intracellular killing activites (Jerrells *et al.*, 1986). As with *R. conorii*, the antirickettsial activities of macrophages

against *R. prowazekii* appear to be regulated primarily by IFN-γ (Wisseman and Waddell, 1983; Turco and Winkler, 1984). More importantly for rickettsial infections, however, these investigators determined that IFN-γ can also restrict multiplication of *R. prowazekii* in several different types of non-immune cells (Turco and Winkler, 1983a, b, c; Wisseman and Waddell, 1983). The mechanism for IFN-induced inhibition of rickettsial replication in fibroblasts and endothelial cells is not known, but is apparently not due to the depletion of tryptophan, as is the case with toxoplasmas and *C. psittaci* (Pfefferkorn, 1984; Byrne *et al.*, 1986; Turco and Winkler, 1984). *R. prowazekii* is also a nonviral inducer of IFN-α and IFN-β during active infections in experimental animals (Kazar, 1966). Unlike the effects of IFN-γ, however, intracellular replication of *R. prowazekii* is not inhibited by IFN-α/β (Turco and Winkler, 1983b). In contrast to the results obtained with *R. prowazekii*, intracellular survival of *R. akari* in fibroblasts is affected by treatment of cells with non-immune IFNs (Kazar *et al.*, 1971).

C. In vivo Infections

1. Experimental animal models

Probably the most striking evidence for the inherent diversity of the rickettsiae is the susceptibility of experimental animals to infection with these agents. Guinea pigs and mice have been used extensively for isolation of rickettsial agents, but the availability of genetically inbred mice facilitated analysis of host factors that may be important in resolution of rickettsial diseases. It came as no surprise to rickettsiologists, who had grappled with rickettsial strain and intrastrain diversity for years, that different rickettsial species manifested different patterns of susceptibility in inbred mouse stocks: some rickettsiae (for example, Karp strain of *R. tsutsugamushi*) were uniformly lethal to mice when injected intraperitoneally (ip); other closely related rickettsiae (Gilliam strain of *R. tsutsugamushi*) induced a pattern of resistance to ip infection that suggested a genetically determined resistance trait (Groves and Osterman, 1978). In fact, the gene for natural resistance to ip infection by Gilliam *R. tsutsugamushi, Ric*, has been mapped to chromosome 5 of the mouse, and is linked to the gene for retinal degeneration (Groves *et al.*, 1980). The resistant allele of this gene has been grafted onto the susceptible C3H/He genetic background, creating a congenic C3H/RV mouse that differs from susceptible C3H/He mice in but a single genetic locus (Darnell *et al.*, 1974). Changing the route of *R. tsutsugamushi* inoculation, however, changes the whole picture of mouse strain suscep-

tibility: both Karp and Gilliam strains administered subcutaneously (sc) produce no detectable disease in the ip-susceptible animals, and will, in fact, protect mice from lethal ip rickettsial challenge with either of the strains; mice given an intravenous (iv) inoculation of Gilliam are resistant to lethal disease (Groves and Osterman, 1978; Jerrells and Osterman, 1982b).

That these differences within rickettsial strains are not a peculiarity of *R. tsutsugamushi* is underscored by studies with a spotted fever group rickettsia, *R. akari*. Analysis of inbred mouse susceptibility to different strains of this rickettsia showed that most strains of *R. akari* did not produce lethal disease when inoculated by any route. Inoculation of the Kaplan strain did, however, produce an interesting pattern of susceptibility that suggested macrophage control of resistance (Anderson and Osterman, 1980; Meltzer and Nacy, 1980): each mouse strain susceptible to lethal *R. akari* disease had a characterised macrophage defect (Boraschi and Meltzer, 1979a, b and c). The route of inoculation of this rickettsia was not critical to the outcome of disease (Anderson and Osterman, 1980). Further analysis of innate resistance to *R. akari* demonstrated that the gene responsible for susceptibility in C3H mice was the *Lps* gene on chromosome 4, a locus that controls not only susceptibility to the lethal effects of bacterial LPS, but also the ability of macrophages to respond to activation agents for intra- and extra-cellular killing effector mechanisms (Anderson and Osterman, 1980; Watson *et al.*, 1977; Ruco *et al.*, 1978; Nacy and Meltzer, 1982). The observation that C3H/HeJ mice are susceptible to lethal infections with two other spotted fever group rickettsiae, *R. conorii* and *R. siberica*, suggests an underlying common mechanism of resistance to this group of micro-organisms (Eisemann *et al.*, 1984).

The A strain mice are also susceptible to the Kaplan strain of *R. akari*, and have a macrophage defect for extracellular killing that is not under *Lps* gene control (Meltzer and Nacy, 1980; Boraschi and Meltzer, 1979b, c). Although macrophages from *R. akari*-infected A/J mice do not develop the capacity to kill tumour cells, a characteristic of their macrophage defect, rickettsial susceptibility could be genetically dissociated from the expression of this macrophage effector activity (Meltzer *et al.*, 1982; Nacy and Meltzer, 1982). The gene(s) that regulate susceptibility to *R. akari* infections in A strain mice are presently being mapped using recombinant inbred mice: preliminary analysis of the data suggests multigenic control.

Despite the availability of congenic pairs of mice whose innate susceptibility to disease is regulated by single genes (C3H/He [Ric^s] and C3H/RV [Ric^r] for *R. tsutsugamushi*; C3H/He [Lps^n] and C3H/HeJ

[Lps^d] for *R. akari*), the host factors that influence resistance or susceptibility to any of the rickettsiae remain undefined. At best, we can say that inflammation and macrophages probably play a role, and that the induction of non-specific immunity early in infection influences the outcome of disease (Jerrells and Osterman, 1981, 1982a, 1982b; Jerrells, 1983; Nacy and Groves, 1981; Nacy and Meltzer, 1982, 1984). Resistance of certain mouse strains to iv-inoculated *R. tsutsugamushi* can be abolished by treatment of mice with agents which destroy tissue macrophages (Jerrells and Osterman, 1982b); at some level, then, expression of resistance is an innate capacity of tissue macrophages. In lethal animal models, however, factors that regulate development of specific antigen recognition by T lymphocytes are most likely to influence resistance: Ia antigen expression, essential for the presentation of antigens, is comparatively downregulated in macrophages of the susceptible animals (Jerrells, 1983), and cells that most efficiently respond to IFN-γ for expression of Ia antigens — the younger peroxidase granule-containing macrophages — are not a major component of inflammatory exudates during lethal rickettsial infections (Nacy and Groves, 1981). The importance of the induction of immune responses for resistance against rickettsial diseases is demonstrated with monoclonal antibodies prepared against IFN-γ. The administration of monoclonal anti-IFN antibodies to resistant animals before infection with *R. conorii* blocks IFN-γ activity and Ia antigen expression, and exacerbates disease: more than 50 per cent of normally resistant mice die of fulminant rickettsial infections (Li, *et al.*, 1987). Whether antibodies administered later in infection could affect development of macrophage killing activities rather than Ia antigen-induced inductive events was not studied. Macrophages from *R. conorii*-infected mice do develop the capacity to eliminate intracellular rickettsiae (Kokorin *et al.*, 1980). That non-specific macrophage-mediated immunity is important in development of resistance to infection with *R. tsutsugamushi* and *R. akari* as well is suggested by protection studies with non-specific macrophage-activating agents (Nacy *et al.*, 1983; Nacy and Meltzer, 1984). In these studies, susceptible animals pretreated with *Mycobacterium bovis* strain BCG or *Propionobacterium acnes* (*C. parvum*) could be protected from lethal disease as long as they were able to respond to the non-specific activation agent with rickettsiacidal macrophages; susceptible mice with additional genetic defects in capacity to generate activated macrophages were not protected. Thus, macrophages appear to play a pivotal role in both the induction of immunity and the performance of effector functions in the expression of innate resistance to rickettsial infections.

2. Human infections

The rickettsiae all cause febrile exanthems, a result of infection of endothelial cells and peripheral vasculitis. Clinical symptoms, morbidity and mortality vary with the different rickettsial strains. Little is known about factors that influence natural resistance or successful resolution of disease with any these organisms. In all cases, intervention by drugs is recommended to reduce mortality: tetracycline is the drug of choice.

IV. SUMMARY

For both the chlamydiae and rickettsiae, the host responses that suppress replication of the organism and the host responses that promote survival of the organism paradoxically overlap. Thus the classic non-specific host defences that facilitate elimination of bacteria in an extracellular environment (i.e. inflammation, phagocytosis) now supply these obligate intracellular parasites with more susceptible cells, and a greater chance for survival. Once inside the cell, the host must now induce extraordinary changes in the intracellular milieu of a number of cell types to eliminate, or at least suppress the replication of, the infecting bacterium. That mortality rates are frequently far less than might be expected is strong testimony to the innovative evolution of both innate and acquired immune responses. Despite evidence for these novel host responses that excite and fascinate the basic researcher, analysis of host defence mechanisms, unfortunately, takes second place to design of optimal drug therapy for any micro-organism susceptible to conventional antibiotics. For both the chlamydiae and rickettsiae, drug therapy works. Only when evidence emerges that therapy is less than completely effective will there be an increase in interest in the precise nature of host-derived factors that contribute to natural resistance. Natural immunity certainly plays a role in resistance to infection, but the mechanisms involved, and the balance between host and parasite-derived factors that influence this naturally occurring resistance, are yet to be determined.

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